October 2005 • Volume 205 • Number 1

Journal of Cellular Physiology

Mammary Gland MMPs

Articles published online in Wiley InterScience,
12 April 2005 - 27 July 2005

WILEY-LISS

JOURNAL OF CELLULAR PHYSIOLOGY 205:123–132 (2005)

Expression Pattern of Four Membrane-Type Matrix Metalloproteinases in the Normal and Diseased Mouse Mammary Gland

LUDMILA SZABOVA, SUSAN S. YAMADA, HENNING BIRKEDAL-HANSEN, AND KENN HOLMBECK*

Craniofacial and Skeletal Diseases Branch, Matrix Metalloproteinase Unit, National Institute of Dental and Craniofacial Research; National Institutes of Health, Bethesda, Maryland

Both mammary gland development and mammary carcinogenesis involve extensive remodeling of the mammary gland extracellular matrix. The expression of four membrane-type matrix metalloproteinases (MT-MMPs) with matrix remodeling potential in development and tumorigenesis was evaluated by in-situ hybridization on mouse mammary gland sections. MT1-MMP and MT3-MMP were found in the mammary stroma mainly around epithelial structures in both developing and mature mammary gland. In contrast, MT2-MMP was found exclusively in the mammary epithelium. Lactating gland expressed none of the examined MT-MMPs. Mammary gland tumors expressed MT1-MMP, MT2-MMP, and MT3-MMP while MT4-MMP was not expressed in any developmental or cancerous stage analyzed here. Our results suggest that MT1-MMP, MT2-MMP, and MT3-MMP may be involved in remodeling of both the normal and diseased mammary gland either directly or indirectly by activation of other MMPs. J. Cell. Physiol. 205: 123–132, 2005. Published 2005 Wiley-Liss, Inc.†

The mammary gland is a highly dynamic organ, which undergoes growth and remodeling during pubertal development and later during postnatal life as the gland adapts to the physiological requirements of lactation. In the mouse, the mammary gland starts to form during embryogenesis at day 10–11 and at the time of birth a small tree of epithelial ducts is established. Under the stimulus of hormones and growth factors, the gland epithelium further develops following the onset of puberty to attain the maturity of the adult virgin gland. At this stage, the gland is fully competent to respond to the hormonal stimuli of pregnancy, which induce morphological and functional changes to support lactation (Hennighausen and Robinson, 1998). Following cessation of lactation, the gland rapidly undergoes remodeling to regain a configuration similar to that of the adult virgin gland. The changes from initial development through pregnancy and involution are associated with substantial morphological transformations of both the branched epithelial tissue and the associated periductal stroma and fat pad. Although the remodeling and morphogenesis of the mammary gland tissue are regulated by both systemic and local hormones and growth factors (Hennighausen and Robinson, 1998; Gouon-Evans et al., 2002; Lin et al., 2002; Marshman and Streuli, 2002; Radisky et al., 2003; Sapi, 2004), the actual structural remodeling of the tissue is ultimately dependent on coordinated proliferation, cell death, synthesis, and removal of the extracellular matrix.

The matrix metalloproteinases (MMPs) constitute a group of zinc endopeptidases capable of cleaving multiple extracellular matrix and basement membrane macromolecules (Brinckerhoff and Matrisian, 2002; Egeblad and Werb, 2002). Several members of the MMP family possess collagenolytic activity and as such may be important for cell migration, proliferation, and remodeling of the collagenous matrices in the mammary gland (Holmbeck et al., 1999; Hotary et al., 2003). Indeed, several lines of evidence pointing in this direction are supported by the observation that many MMPs are expressed in the mammary gland (Lund et al., 1996; Rudolph-Owen and Matrisian, 1998; Djonov et al., 2001; Wiseman et al., 2003). The significance of these observations, however, is difficult to determine. A number of mouse strains deficient for individual MMPs have been developed and have provided an opportunity to study the role of specific MMP deficiencies on mammary gland development. These mouse models demonstrate that some secreted MMPs indeed participate in branching morphogenesis and involution but none of these gene deficiencies appear to interfere with the lactating function of mammary gland (Itoh et al., 1997; Mudgett et al., 1998; Itoh et al., 1999; Caterina et al., 2000; Caterina et al., 2002). Here, we extend the analysis of MMP expression in the mammary gland to include four membrane-type MMPs (MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP) in the normal mammary gland during different developmental stages as well as in mammary adenocarcinoma. We report here that three of the four membrane-type matrix metalloproteinases (MT-MMPs) are expressed in a distinct temporal and spatial manner suggesting that their catalytic activity likely contributes to both the development and subsequent remodeling of the gland during the reproductive cycle and during tumor development.

Abbreviations: MMP, matrix metalloproteinase; MT1-MMP, membrane-type 1 matrix metalloproteinase; MT2-MMP, membrane-type 2 matrix metalloproteinase; MT3-MMP, membrane-type 3 matrix metalloproteinase; MT4-MMP, membrane-type 4 matrix metalloproteinase.

Contract grant sponsor: NIDCR/DIR; Contract grant number: Z01DE000676.

*Correspondence to: Kenn Holmbeck, NIDCR/NIH, Bldg. 30, Rm. 4A400, MSC4380, 30 Convent Drive, Bethesda, MD 20892. E-mail: kenn.holmbeck@nih.gov

Received 17 September 2004; Accepted 18 January 2005

DOI: 10.1002/jcp.20385

PUBLISHED 2005 WILEY-LISS, INC.

TABLE 1. Probe information

	Position	GenBank accession
MT1-MMP	291–902 bp	X83536
MT2-MMP	180–1123 bp	D86332
MT3-MMP	163–1614 bp	AF282844
MT4-MMP	782–1520 bp	AB021224
β-actin	762–1837	X03672

For the generation of hybridization probes, DNA fragments in the given position on the appropriate MT-MMP GenBank accession number were used.

MATERIALS AND METHODS

Tissue processing

All animals utilized in this experiment were housed and handled according to animal study proposals approved by the National Institute of Dental and Craniofacial Research Animal Care and Use Committee. Mammary glands were harvested from FVBN/J female mice at various ages, fixed overnight in 4% formaldehyde in PBS, washed in PBS, embedded in paraffin, and sectioned at 6 μm. Slides were processed either for hematoxylin/eosin staining, Masson's trichrome staining, or in situ hybridization.

Probes

To generate hybridization probes for MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP, DNA fragments generated by PCR or by restriction digest (Table 1) were cloned into either the pCRII TOPO TA cloning vector (Invitrogen, Carlsbad, CA) or pBluescript SK+ (Stratagene, La Jolla, CA). The plasmids were transcribed using SP6, T3, or T7 RNA polymerases to generate the sense and antisense RNA probes.

In situ hybridization

In situ hybridization was performed as previously described (Blavier and DeClerck, 1997). Deparaffinized sections were treated with proteinase K (5 μg/ml), post fixed in 4% formaldehyde in PBS, acetylated in triethanolamine hydrochloride/acetic anhydride, washed in PBS, dehydrated, and air-dried. The sections were hybridized at 50°C to an RNA probe radiolabeled with [α-^{33}P] UTP (DuPont NEN, Boston, MA). For each antisense probe tested, a sense probe was also used as negative control. After hybridization, the sections were washed, coated with an autoradiographic emulsion (LM-1, Amersham Biosciences, Little Chalfont, UK) and exposed for 5 days at 4°C. After exposure, the slides were developed and counterstained with Mayer's hematoxylin.

Northern blot analysis

Total RNA was fractioned on formaldehyde agarose gel, transferred, and immobilized onto nylon membranes and MT-MMP mRNA was detected by hybridization to ^{32}P labeled probes using QuikHyb Hybridization Solution (Stratagene, La Jolla, CA) as recommended by the manufacturer. Separate gels and membranes were prepared for use with each of the MT-MMP probes. Each membrane, after initial hybridization to the MT-MMP probe (Table 1), was stripped and hybridized to a mouse beta actin probe (Table 1) to normalize for the loaded RNA quantity.

Mammary gland whole mounts

For visualization of whole glands, the tissue was mounted onto glass slides, fixed in Carnoy's fixative, rehydrated, stained with carmine alum, and after subsequent dehydration cleared of fat as described previously (Ip and Asch, 2000).

RESULTS

To evaluate the expression of MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP in mouse mammary gland, we performed in situ hybridization on tissues from various developmental stages as well as diseased tissue. These results are summarized in Table 2.

In situ hybridization with an MT4-MMP specific probe on mammary gland sections of different developmental stages and mammary gland tumor sections did not result in any detectable signal (data not shown). To validate the MT4-MMP probe specificity and efficiency, mouse brain sections were tested for expression (data not shown). This experiment demonstrated that the probe efficiently detected MT4-MMP messenger RNA, thus confirming that the lack of detectable signal in mammary gland was due to the absence of MT4-MMP expression rather than being the result of an inefficient probe.

MT-MMP expression in prepubertal mammary gland

At birth, the mammary gland epithelium is localized in a peripheral part of the mammary fat pad as a small epithelial tree. Epithelial ducts in the 4-day-old gland can be observed in a small area near the nipple (Fig. 1A, arrowheads; 1B, arrows) while the rest of the mammary tissue consists of the fat pad. MT1-MMP was expressed exclusively in the stroma of the 4-day-old mammary gland (Fig. 1D, arrows and arrowheads), particularly in the collagen-rich area around epithelial ducts (Fig. 1C, arrows). Throughout the fat pad, a somewhat weaker signal specific for MT1-MMP was detected suggesting that MT1-MMP is also expressed in adipogenic tissue, albeit at a lower level. MT2-MMP, however, was expressed exclusively in the mammary ductal epithelium and not in the surrounding stroma or the fat pad (Fig. 1G, arrows). An adjacent section hybridized to a probe specific for MT3-MMP messenger RNA demonstrated a robust signal in the peri-ductal stroma (Fig. 1J, arrows). The expression pattern of MT3-MMP was somewhat similar to that obtained with an MT1-MMP-specific probe.

MT-MMP expression in adult virgin mammary gland

The growth and development of the mammary epithelium continues after the onset of puberty at the age of 4–5 weeks. Under the influence of reproductive hormones, the mammary epithelium starts to grow further into the fat pad. As a result, the adult virgin gland is ultimately filled with epithelial ducts penetrating the

TABLE 2. MT-MMP expression profile summary

	MT1-MMP	MT2-MMP	MT3-MMP	MT4-MMP
Prepubertal mammary gland	S	E	S	0
Adult mammary gland	S	E	S	0
Pregnant mammary gland	S	E	S	0
Lactating mammary gland	0	0	0	0
Involuting mammary gland	S	E	S	0
Mammary gland tumor	S	E	S	0

Summary of the MT-MMP expression pattern in the main compartments of the mouse mammary gland. S, mammary gland stroma; E, mammary gland epithelium, 0, not detected.

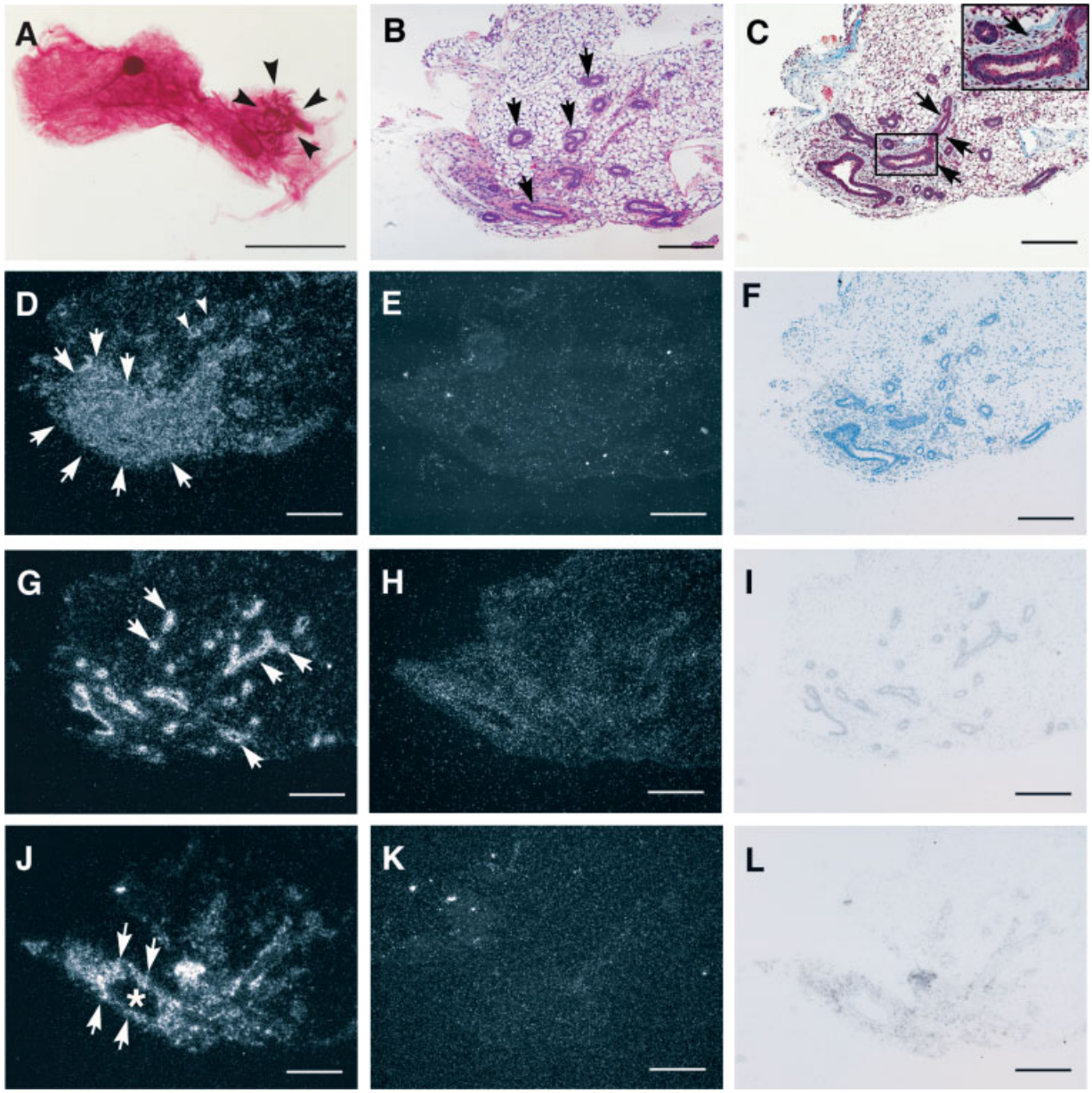

Fig. 1. Whole mount staining and in situ hybridization on a 4-day-old mammary gland. **A**: Whole mount of mammary gland of a 4-day-old mouse showing a small epithelial tree on the periphery (arrowheads). Scale bar represents 2 mm (**B**) H & E stained section of 4-day-old mouse mammary gland with abundant epithelial ducts (arrows) surrounded by collagen layer (**C**, arrows) as showed by Masson's trichrome staining (collagen staining blue). The framed area is enlarged in the inset. **D**: Serial section hybridized to antisense MT1-MMP probe shows strong staining of the stroma in the area rich in epithelial ducts (arrows) and around isolated ducts (arrowheads). **G**: MT2-MMP is strongly expressed in epithelial ducts (arrows), (**J**) and MT3-MMP is expressed in the stroma (arrows) surrounding ducts (asterisk). **D**, **G**, and **J**: Antisense probes for MT1-MMP, MT2-MMP, and MT3-MMP, respectively (dark field). (**F**, **I**, and **L**) the same sections in bright field; (**E**, **H**, and **K**) sense counterparts to (**D**, **G**, and **J**) in dark field. Scale bar in (**B–L**) represents 100 μm.

entire fat pad (Fig. 2A). The MT-MMP expression patterns in 60-day-old virgin mice were chosen as representative of the adult stage of mammary gland development. At this time, epithelial ducts had completely filled in the mammary gland fat pad (Fig. 2B). The expression pattern of MT1-MMP was very similar to that observed at 4 days, but more confined to the periductal region in the gland (Fig. 2D, arrows) and to a lesser extent to the fat pad at large (Fig. 2D, arrow heads). The longitudinal section of the branching ducts depicted in Figure 2G (arrows), as well as cross sections of some smaller ducts (Fig. 2G, arrow heads) displayed a robust and highly restricted expression of MT2-MMP in mammary epithelial cells. MT3-MMP, on the other hand, was expressed in the stroma surrounding epithelial ducts, much in the same manner as the signal for MT1-MMP, although with minor differences in the actual distribution of staining (Fig. 2J, arrows).

The most highly proliferating regions of the epithelium, the terminal end buds, displayed a pattern of expression that is consistent with the expression pattern in ducts proper (data not shown). In addition, we did see expression suggesting that some epithelial cells may express MT1-MMP and MT3-MMP. However, the evaluation of these observations was complicated by our inability to clearly differentiate between epithelial and

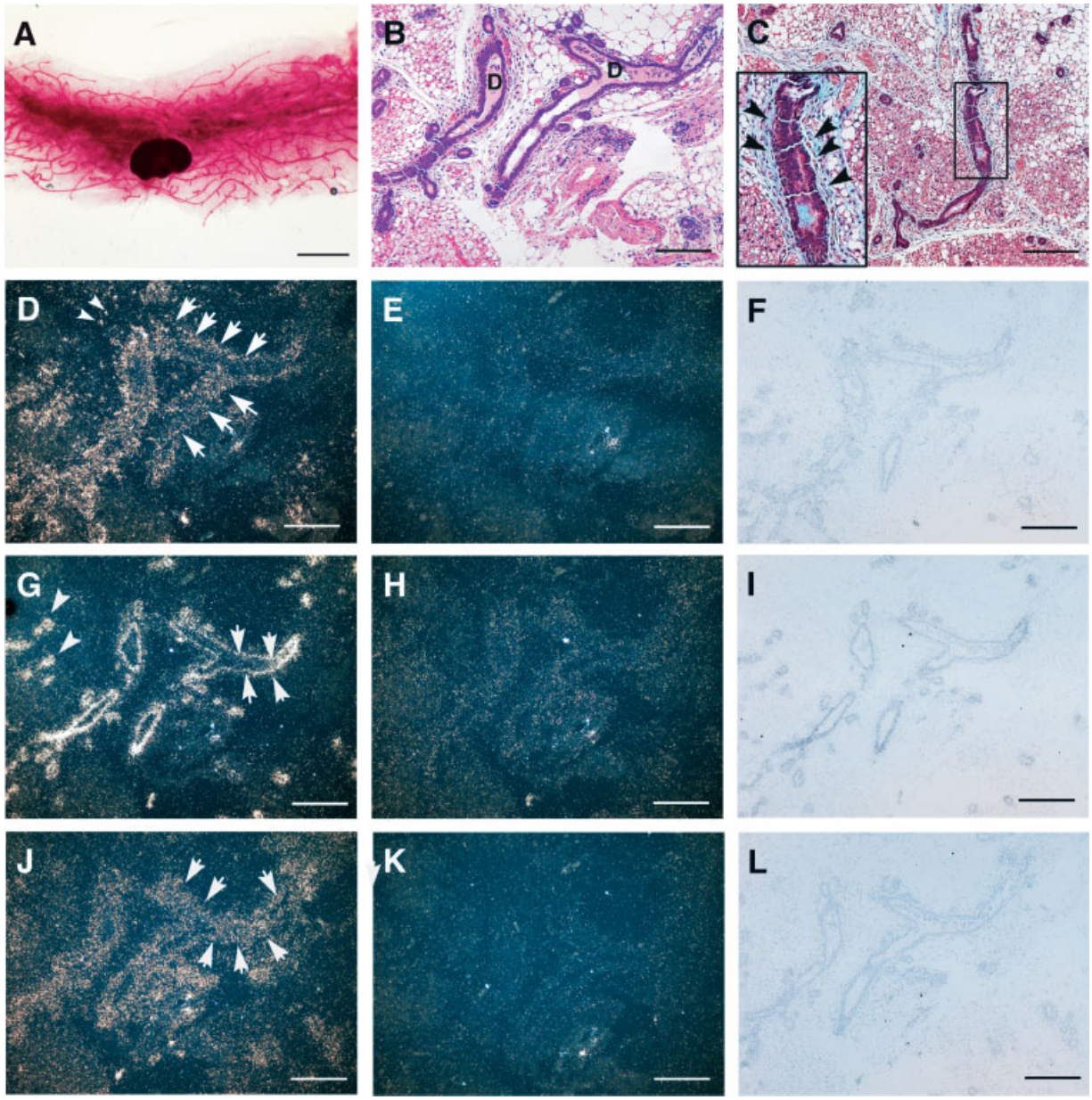

Fig. 2. Whole mount staining and in situ hybridization on a 60-day-old virgin mammary gland. **A**: Whole mount of mammary gland of a 60-day-old mouse. Mammary epithelium fills up the entire fat pad. Scale bar represents 2 mm. **B**: H & E stained section of 60-day-old virgin mouse mammary gland with longitudinal section of the branching duct [D], (**C**) Masson's trichrome staining of the same gland shows collagen layer (blue) around longitudinal section of the duct (arrows). The framed area is enlarged in the inset. **D**: Serial section hybridized to MT1-MMP antisense probe shows strong staining in the stroma around ducts (arrows) and in the stroma of the fat pad (arrowheads), (**G**) MT2-MMP specific probe labels the epithelium of the ducts (arrows for longitudinal section of the duct, arrowheads for the cross section of the ducts), (**J**) MT3-MMP is localized in the stroma around ducts (arrows). **D**, **G**, and **J**: Antisense probes for MT1-MMP, MT2-MMP, and MT3-MMP, respectively (dark field), (**F**, **I**, and **L**) the same sections (bright field); (**E**, **H**, and **K**) sense counterparts to (**D**, **G**, and **J**) in dark field. Scale bar in (**B–L**) represents 100 μm.

stromal cells in these densely packed structures. Based on the tightly restricted expression of MT2-MMP in the epithelium and the resulting staining pattern, we lean towards the interpretation that MT1-MMP and MT3-MMP may, at the most, be expressed in a small subset of epithelial cells, if at all.

MT-MMP expression in pregnant mammary gland

The mammary gland further undergoes extensive changes during pregnancy, when epithelial ducts form multiple branches at the end of which lobules are formed (Fig. 3A,B). A cross section of the pregnant gland hybridized to an MT1-MMP specific probe showed clusters of forming lobules where MT1-MMP was expressed mainly in the stroma surrounding lobules (Fig. 3D, arrows), as well as in the stroma of the fat pad (Fig. 3D, arrow heads). As in the early developmental stages and the adult virgin gland, MT2-MMP-specific hybridization signal was present exclusively in the epithelial cells of the lobules (Fig. 3G, arrows). A signal specific for MT3-MMP could be detected in the stroma of the pregnant gland, however, at modest levels (Fig. 3J). Repeated hybridization experiments on different pregnant mammary gland specimens consistently resulted in the detec-

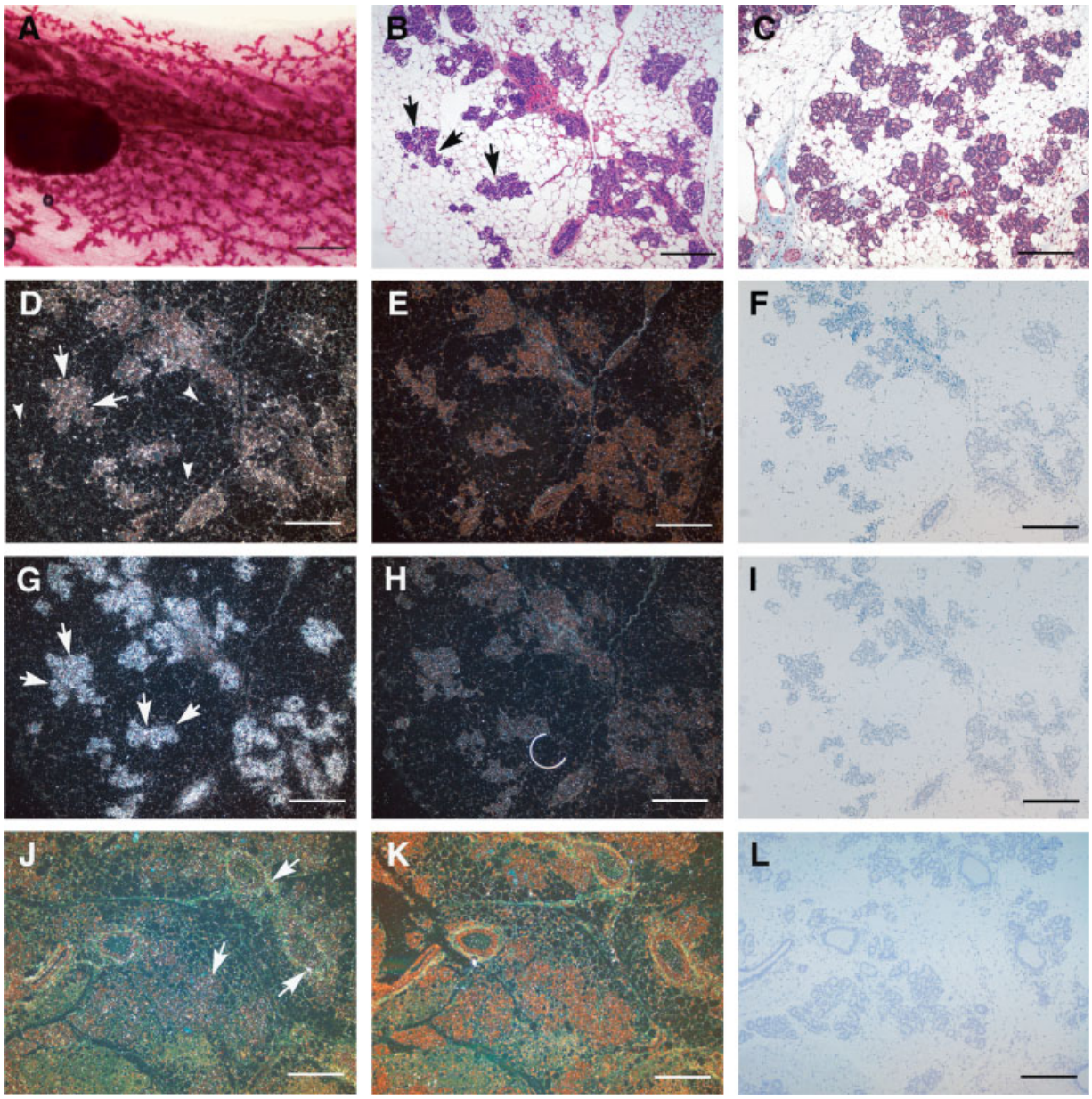

Fig. 3. Whole mount staining and in situ hybridization on a mammary gland from a pregnant mouse. **A**: Whole mount of mammary gland of a pregnant mouse, with extensive branching and lobule formation. Scale bar represents 2 mm. **B**: H & E stained section of 13-day pregnant mouse mammary gland with developing lobules with arrows pointing to clusters of lobules. **C**: Masson's trichrome staining. **D**: Serial section hybridized to MT1-MMP antisense probe shows expression in the stroma proximal to lobular structures (arrows) and in the fat pad (arrowheads). **G**: The MT2-MMP antisense probe labels epithelial lobules (arrows), (**J**) MT3-MMP expression in the stroma around ducts and lobules. **D**, **G**, and **J**: Antisense probes for MT1-MMP, MT2-MMP, MT3-MMP, respectively (dark field), (**F**, **I**, and **L)** the same sections (bright field); (**E**, **H**, and **K)** sense counterparts to (**D**, **G**, and **J)** in dark field. Scale bar in (**B–L**) represents 100 μm.

tion of low levels of MT3-MMP, which led us to conclude that the finding was true.

MT-MMP expression in lactating mammary gland

During lactation, the gland consists mainly of secreting lobules producing milk (Fig. 4A,B). At this stage, we were unable to detect expression of MT1-MMP, MT2-MMP, and MT3-MMP in the mammary gland (Fig. 4D,G,J). To confirm this result, we harvested mammary glands from all stages and performed Northern blot analysis with the same probes used for in situ hybridizations (Table 2). This analysis demonstrated that the weakest signal was consistently detected during lactation and thus confirmed our previous observation (Fig. 7).

MT-MMP expression in involuting mammary gland

Weaning of the pups from the lactating female induces involution of the mammary gland (Fig. 5A). This dramatic tissue remodeling process is characterized by a total loss of the lactation-supporting architecture, facilitated by apoptosis and massive matrix remodeling of both mammary epithelium and the associated stroma. The resulting fully involuted gland is thus very similar to the virgin adult gland and consists predominantly of epithelial ducts. In contrast to the steady state lactation

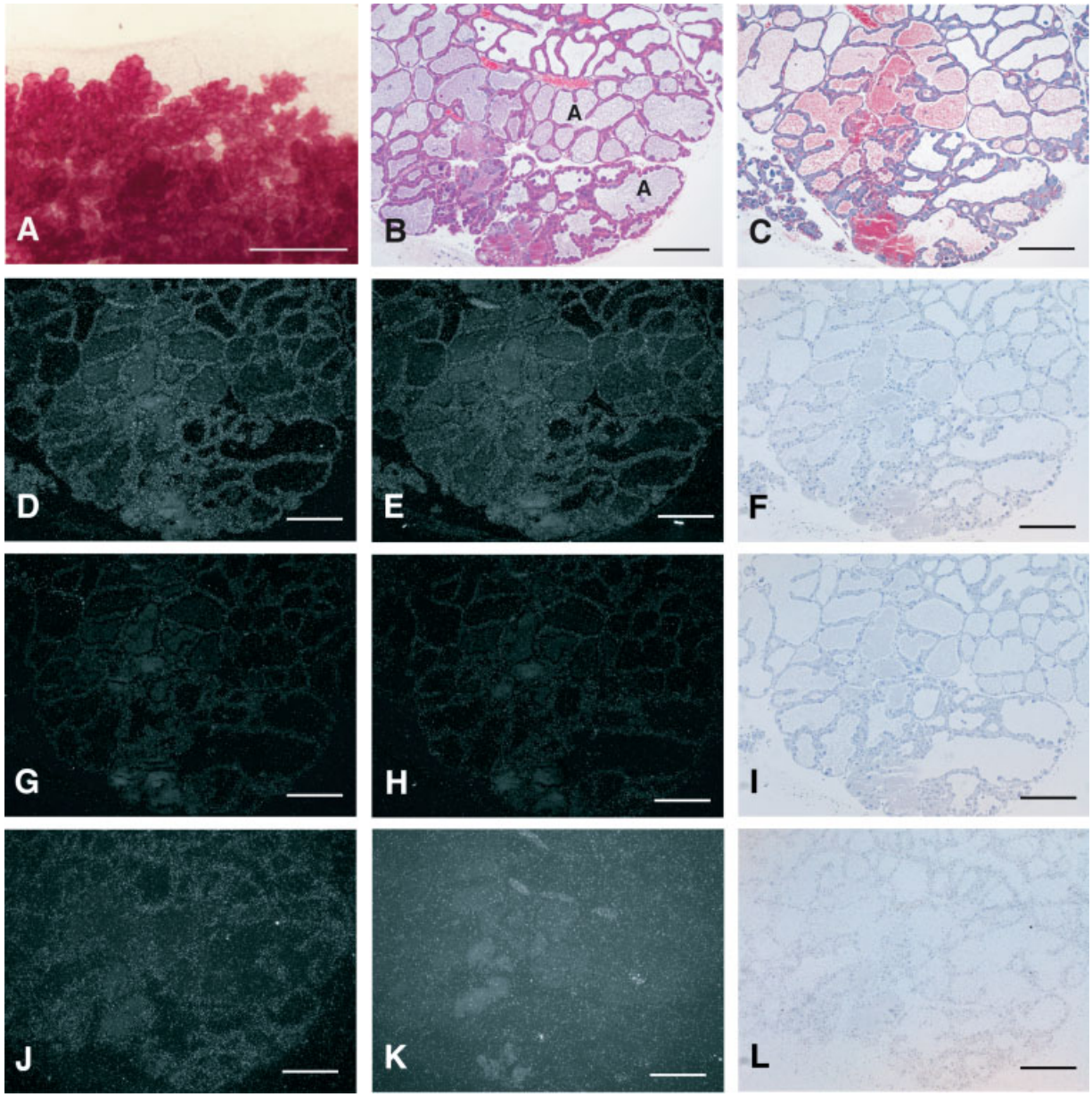

Fig. 4. Whole mount staining and in situ hybridization on a lactating mammary gland. **A**: Whole mount stain of a lactating mammary gland showing lobules filled with milk. Scale bar in (A) represents 1 mm. **B**: H & E stained section of a lactating mouse mammary gland composed of lobules [A] filled with milk. **C**: Masson's trichrome staining reveals almost no collagen present between lobular structures. **D**, **G**, and **J**: Serial sections hybridized to MT1-MMP, MT2-MMP, and MT3-MMP probes, respectively, (dark field) show that none of these MT-MMPs were present in the lactating gland, (**F**, **I**, and **L)** the same sections shown in bright field; (**E**, **H**, and **K)** sense counterparts to (**D**, **G**, and **J)** in dark field. Scale bar in (**B–L**) represents 100 μm.

architecture of the mammary gland, the involution stage is associated with re-emergence of expression of all the MT-MMPs present in earlier stages. Figure 5B demonstrates a cross section of the involuting gland (5 h after weaning at the 21st day of lactation) with a duct and collapsed lobules. MT1-MMP was highly expressed in the stroma around the collapsed lobules. In contrast to the MT1-MMP expression observed at earlier time points, the expression in the involuting gland was more punctate yet outlined the remodeling epithelial structures well (Fig. 5D, arrows and arrow heads). MT2-MMP, as in previous time points, was expressed exclusively in the epithelial cells of ducts and involuting lobules (Fig. 5G, arrows and arrowheads). The MT3-MMP expression in the involuting gland had a punctate appearance similar to that of the MT1-MMP expression (Fig. 5J, arrows).

MT-MMP expression in mammary tumors

The extensive changes in the mammary gland during growth and reproductive cycles are part of normal development. However, mammary tissue undergoes a distinctive remodeling process during the growth of mammary tumors. Mice transgenic for the polyoma virus middle T antigen under the control of the MMTV promoter (PyMT mice) develop mammary adenocarcinomas due to expression of the T-antigen in the mammary gland epithelium (Guy et al., 1992). This mouse mammary gland carcinoma model represents a suitable model for human ductal mammary adenocarcinoma

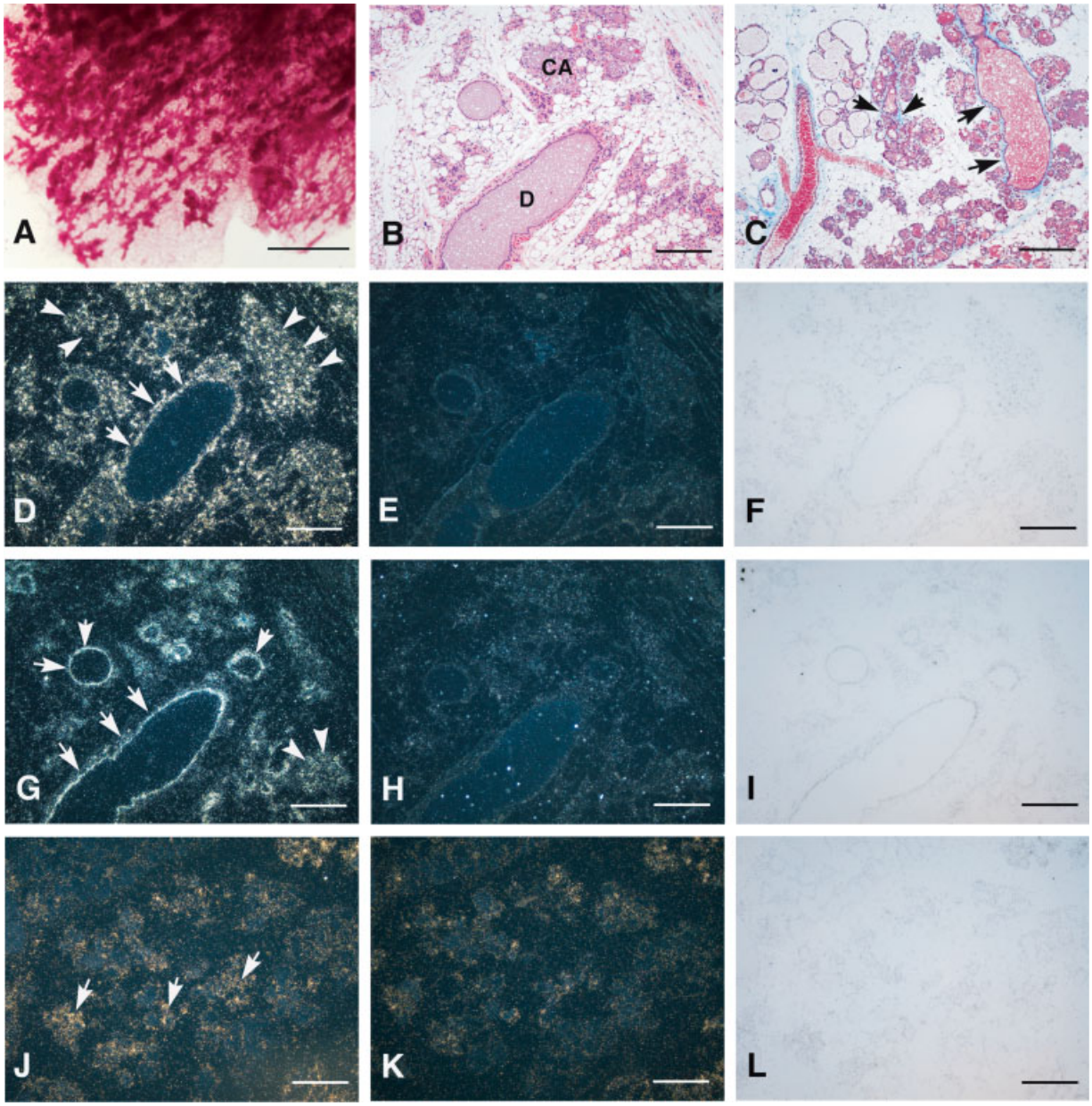

Fig. 5. Whole mount staining and in situ hybridization on an involuting mammary gland. **A**: Whole mount of involuting mouse mammary gland. Scale bar represents 1 mm. **B**: H & E stained section of involuting mouse mammary gland. The gland was harvested 6 days after weaning. [D] indicates duct and [CA] collapsed lobules. **C**: Masson's trichrome staining, arrows pointing to collagen abundant areas (blue stain). **D**: Serial section hybridized to MT1-MMP antisense probe shows labeling of stroma around the ducts (arrows) and in the region of collapsed lobules (arrowheads). **G**: MT2-MMP antisense probe labels epithelial cells of the ducts (arrows) and in collapsed lobules (arrowheads). **J**: MT3-MMP expression is weak and localized to the stroma around collapsed lobules (arrows). **D**, **G**, and **J**: Antisense probes for MT1-MMP, MT2-MMP, MT3-MMP, respectively (dark field), (**F**, **I**, and **L)** the same sections (bright field); (**E**, **H**, and **K)** sense counterparts to (**D**, **G**, and **J)** in dark field; (**J**, **K**, and **L)** showing a different specimen than in the preceding parts. Scale bar in (**B–L**) represents 100 μm.

(Lin et al., 2003). Onset of neoplasia in this model can be detected as early as 4–6 weeks as epithelial hyperplasia. At 7–8 weeks female mice have palpable tumors, which continue to grow and eventually affect all glands. Figure 6A represents a cross section of the tumor from a PyMT mouse (60 days old) with cyst-like structures surrounded by an epithelial cell layer. MT1-MMP was expressed in the tumor stroma around globular cystic structures, especially in close proximity to the epithelial layer (Fig. 6D, arrows). These areas also contained large amounts of collagen as indicated by Masson's trichrome staining (Fig. 6B,C, arrows). In the tumors, MT2-MMP expression was observed in epithelial cells as in the normal developing gland (Fig. 6G, arrows), while the surrounding stroma demonstrated some expression of MT3-MMP (Fig. 6I).

DISCUSSION

We demonstrate here that mammary development in its various phases is associated with expression of at least three membrane-associated MMPs, namely MT1-MMP, MT2-MMP, and MT3-MMP. MT4-MMP does not appear to be expressed at detectable levels at any stage. While MT1-, MT2-, and MT3-MMP are closely related in primary molecular structure, we note that they display different spatial and temporal patterns of expression.

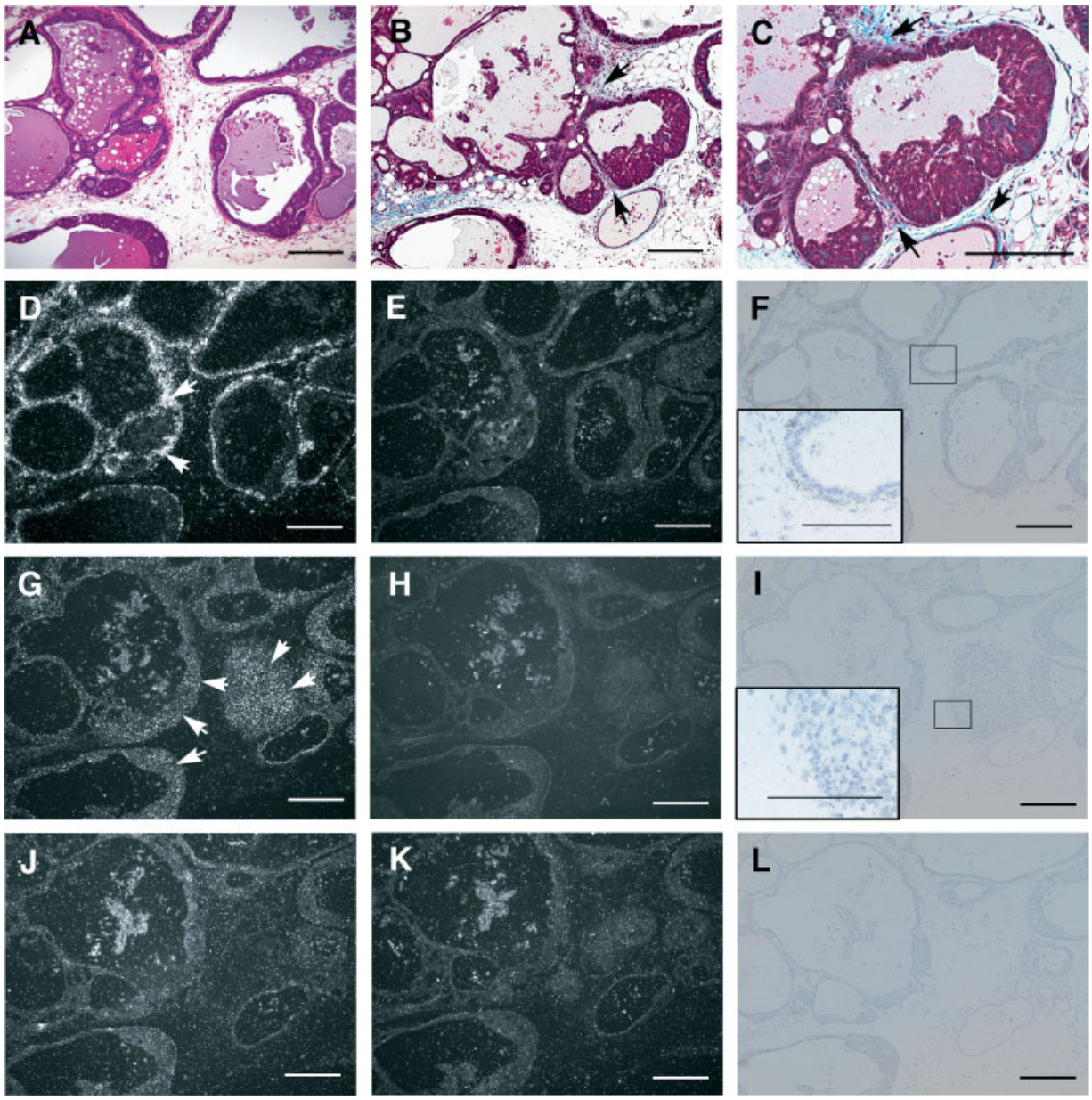

Fig. 6. In situ hybridization on mammary gland with adenocarcinoma. H & E stained section of the mouse mammary gland adenocarcinoma **A**: Masson's trichrome staining (**B**), arrows pointing to the collagenous layer surrounding tumor acini (enlarged in **C**). **D**: Serial section hybridized to MT1-MMP antisense probe shows labeling of the stroma in the near proximity to the epithelial layer of tumor acini (arrows). **G**: MT2-MMP is expressed in the epithelial layer (arrows). **J**: MT3-MMP expression is weak and localized to the stroma. **D**, **G**, and **J**: Antisense probes for MT1-MMP, MT2-MMP, MT3-MMP, respectively (dark field), (**F**, **I**, and **L)** the same sections (bright field); (**E**, **H**, and **K)** sense counterparts to (**D**, **G**, and **J)** (dark field). Insets in (**F**) and (**I**) demonstrates labeling of stromal and epithelial cells, respectively. Scale bar represents 100 μm.

The most abundant component of the mammary gland extracellular matrix is type I collagen, produced not only by resident fibroblasts of the peri-ductal stroma and also by cells scattered among adipocytes in the fat pad (Hovey et al., 1999). In order to allow epithelial cell proliferation, the supporting collagenous stroma, (Figs. 1C and 2C) must be remodeled most likely by collagenase-type proteinases (Holmbeck et al., 1999). Several members of the MMP family possess potent collagenase activity in vitro, in addition to activity against other stromal and basement membrane molecules. It is assumed that MMPs play important roles in the remodeling and degradation of mammary stroma. Previous work has detailed the expression of secreted MMPs in mammary gland development. MMP-3 (stromelysin-1), MMP-2 (gelatinase A), and MMP-9 (gelatinase B) are present in the mammary tissue (Wiseman et al., 2003) and play a role in branching morphogenesis and in involution. Some of the mouse strains deficient for specific secreted MMPs display altered mammary gland morphology or involution with no apparent impairment of lactation. Less attention has been paid to the expression and possible role of membrane-type MMPs in the mammary gland.

The role of MT1-MMP in the timely remodeling of "soft" collagen type I rich matrices has previously been explored in detail (Holmbeck et al., 1999; Hotary et al., 2002; Holmbeck et al., 2003; Hotary et al., 2003). In accordance with its role as the cell-associated collagen-cleaving enzyme, we have observed that mammary

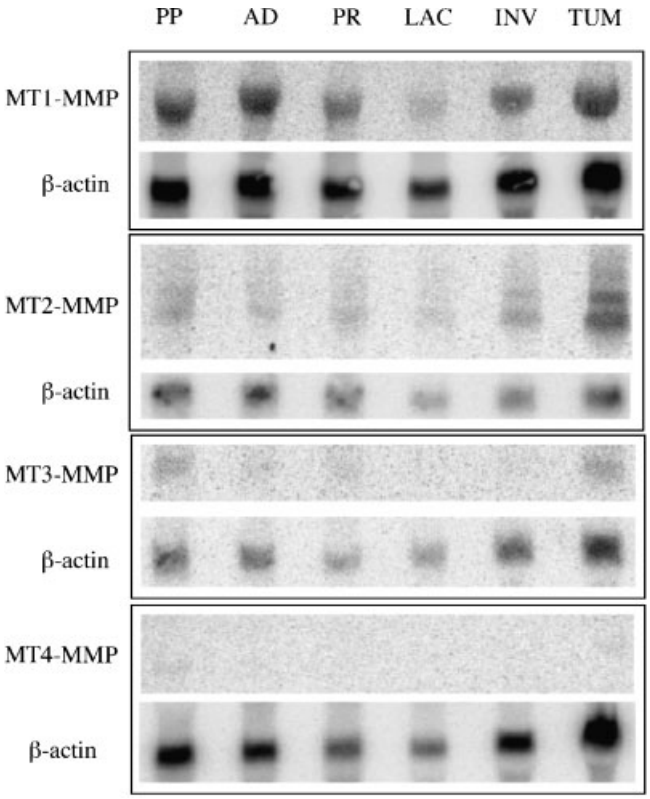

Fig. 7. Northern blot analysis of MT-MMPs expression in mouse mammary glands. PP, prepubertal gland (3 days old); AD, adult gland (60 days old); PR, pregnant gland (13 days pregnant); LAC, lactating gland; INV, involuting gland (3 days after weaning); TUM, mammary gland tumor. The upper portion of each part shows the result of hybridization to the indicated MT-MMPs, the lower portion of each part shows hybridizaton of the same membrane to a mouse β-actin probe.

stroma in adult MT1-MMP-deficient mice displays overt signs of fibrosis (unpublished data). Together with the strong expression observed here in the collagen-rich periductal stroma during initial development, pregnancy, and involution, these findings suggest that MT1-MMP may also function as a collagenase in the mammary gland. It is of note that during the relatively steady state phase of lactation, none of the enzymes are expressed and apparently the remodeling processes required during development, growth, and involution of the gland are temporarily inactivated. The expression of these proteases is temporally associated with periods of synthesis, removal, and pruning of the extracellular matrix in order to facilitate cell behavior such as proliferation, migration, and apoptosis (Pilcher et al., 1997a; Parks, 1999). We have previously observed that MT1-MMP-dependent matrix degradation facilitates apoptotic cell demise in mesenchymal cells as well as steady state turnover of matrix in response to growth requirements (Holmbeck et al., 2003).

A very limited body of literature exists on the function and expression pattern of MT2-MMP. Surprisingly, in the mammary gland the expression of this protease is entirely restricted to the epithelial compartment. To our knowledge this is the first report of an MT-type MMP restricted to epithelial cells. This observation raises the question of whether MT2-MMP is the epithelial membrane-bound counterpart to MT1-MMP, which, in our experience, appears restricted solely to mesenchymal cells. As is the case for MT1-MMP, expression of MT2-MMP is observed only in stages of morphological change; that is, when cells migrate, die, or proliferate with the coordinated remodeling of resident extracellular matrix. Epithelial cell migration on collagenous matrices is known to require collagenolytic activity in vitro (Pilcher et al., 1997b; Holmbeck et al., 1999), and collagenase-resistant mice display significantly delayed wound reepithelialization as do mice treated with MMP inhibitors (Lund et al., 1999; Beare et al., 2003). Moreover, it has been reported that MT2-MMP confers otherwise collagenase-insufficient cells with the ability to proliferate in vitro (Hotary et al., 2003). Based on these observations it is tempting to speculate that MT2-MMP either directly or indirectly, for instance via pro-MMP2 activation, may confer on mammary epithelial cells a proteolytic capacity to modulate their environment in response to hormonal stimuli.

The distribution of MT3-MMP in mammary gland stroma at least partially overlaps that of MT1-MMP. Like MT2-MMP, MT3-MMP has previously been demonstrated to be able to activate pro-MMP2 and it remains possible that MT3-MMP may function in this capacity in the mammary gland.

Despite cancerous transformation of the gland, MT1-MMP remained confined to the stroma as in the normal gland. Likewise, MT2-MMP was expressed exclusively by epithelial cells both in the normal mammary gland and in adenocarcinoma. Elevated expression of MT1-MMP has been reported in various human carcinomas (Ueno et al., 1997; Jones et al., 1999; Dalberg et al., 2000; Bisson et al., 2003), whereas MT2-MMP and MT3-MMP are not often observed in tumor tissue (Polette and Birembaut, 1998). We suggest that the discrepancy between human tumors/tumor cell lines expressing MT1-MMP and our consistent failure to detect epithelial cell expression in the mouse may reflect both cellular properties attained by in vitro culture of human cells as well as species specific expression differences in vivo. We note, with some interest, the lack of MT4-MMP expression in mouse mammary adenocarcinoma. MT4-MMP was originally isolated from a human breast carcinoma source (Puente et al., 1996) and the absence of MT4-MMP in the mouse tumor demonstrates the heterogeneity of different malignancies and also illustrates that the expression pattern presented here cannot be considered a benchmark for malignancy-associated MMP expression.

In summary, we find expression of MT1-MMP and MT3-MMP in the mammary gland stroma while MT2-MMP is expressed exclusively in the mammary epithelium. It is of note that none of the MT-MMPs, according to in situ hybridization results, is expressed during lactation. We confirmed this result by Northern blot analysis of total mammary tissue, which demonstrated very low levels of message for the target molecules. This can be attributed to the expression in extraglandular tissues inadvertently harvested with the gland. We note, for instance, that lymphatic arteries express abundant MT2-MMP message. In addition, the peripheral mammary connective tissue constitutively expresses MT1-MMP. Mammary adenocarcinoma displays expression of MT1-MMP and MT3-MMP in the stromal compartment while MT2-MMP is restricted to the epithelial cells as found in the normal mammary tissue.

ACKNOWLEDGMENTS

We thank Dr. Thomas Bugge for critical reading of the manuscript and for his valuable comments and suggestions.

LITERATURE CITED

Beare AH, O'Kane S, Krane SM, Ferguson MW. 2003. Severely impaired wound healing in the collagenase-resistant mouse. J Invest Dermatol 120(1):153–163.

Bisson C, Blacher S, Polette M, Blanc JF, Kebers F, Desreux J, Tetu B, Rosenbaum J, Foidart JM, Birembaut P, Noel A. 2003. Restricted expression of membrane type 1-matrix metalloproteinase by myofibroblasts adjacent to human breast cancer cells. Int J Cancer 105(1):7–13.

Blavier L, DeClerck YA. 1997. Tissue inhibitor of metalloproteinases-2 is expressed in the interstitial matrix in adult mouse organs and during embryonic development. Mol Biol Cell 8(8):1513–1527.

Brinckerhoff CE, Matrisian LM. 2002. Matrix metalloproteinases: A tail of a frog that became a prince. Nat Rev Mol Cell Biol 3(3):207–214.

Caterina JJ, Yamada S, Caterina NC, Longenecker G, Holmback K, Shi J, Yermovsky AE, Engler JA, Birkedal-Hansen H. 2000. Inactivating mutation of the mouse tissue inhibitor of metalloproteinases-2(Timp-2) gene alters proMMP-2 activation. J Biol Chem 275(34):26416–26422.

Caterina JJ, Skobe Z, Shi J, Ding Y, Simmer JP, Birkedal-Hansen H, Bartlett JD. 2002. Enamelysin (matrix metalloproteinase 20)-deficient mice display an amelogenesis imperfecta phenotype. J Biol Chem 277(51):49598–49604.

Dalberg K, Eriksson E, Enberg U, Kjellman M, Backdahl M. 2000. Gelatinase A, membrane type 1 matrix metalloproteinase, and extracellular matrix metalloproteinase inducer mRNA expression: Correlation with invasive growth of breast cancer. World J Surg 24(3):334–340.

Djonov V, Hogger K, Sedlacek R, Laissue J, Draeger A. 2001. MMP-19: Cellular localization of a novel metalloproteinase within normal breast tissue and mammary gland tumours. J Pathol 195(2):147–155.

Egeblad M, Werb Z. 2002. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2(3):161–174.

Gouon-Evans V, Lin EY, Pollard JW. 2002. Requirement of macrophages and eosinophils and their cytokines/chemokines for mammary gland development. Breast Cancer Res 4(4):155–164.

Guy CT, Cardiff RD, Muller WJ. 1992. Induction of mammary tumors by expression of polyomavirus middle T oncogene: A transgenic mouse model for metastatic disease. Mol Cell Biol 12(3):954–961.

Hennighausen L, Robinson GW. 1998. Think globally, act locally: The making of a mouse mammary gland. Genes Dev 12(4):449–455.

Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, Ward JM, Birkedal-Hansen H. 1999. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell 99(1):81–92.

Holmbeck K, Bianco P, Chrysovergis K, Yamada S, Birkedal-Hansen H. 2003. MT1-MMP-dependent, apoptotic remodeling of unmineralized cartilage: A critical process in skeletal growth. J Cell Biol 163(3):661–671.

Hotary KB, Yana I, Sabeh F, Li XY, Holmbeck K, Birkedal-Hansen H, Allen ED, Hiraoka N, Weiss SJ. 2002. Matrix metalloproteinases (MMPs) regulate fibrin-invasive activity via MT1-MMP-dependent and -independent processes. J Exp Med 195(3):295–308.

Hotary KB, Allen ED, Brooks PC, Datta NS, Long MW, Weiss SJ. 2003. Membrane type I matrix metalloproteinase usurps tumor growth control imposed by the three-dimensional extracellular matrix. Cell 114(1):33–45.

Hovey RC, McFadden TB, Akers RM. 1999. Regulation of mammary gland growth and morphogenesis by the mammary fat pad: A species comparison. J Mammary Gland Biol Neoplasia 4(1):53–68.

Ip MM, Asch BB. 2000. Methods in mammary gland biology and breast cancer research. New York: Kluwer Academic/Plenum Publishers, 329p.

Itoh T, Ikeda T, Gomi H, Nakao S, Suzuki T, Itohara S. 1997. Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice. J Biol Chem 272(36):22389–22392.

Itoh T, Tanioka M, Matsuda H, Nishimoto H, Yoshioka T, Suzuki R, Uehira M. 1999. Experimental metastasis is suppressed in MMP-9-deficient mice. Clin Exp Metastasis 17(2):177–181.

Jones JL, Glynn P, Walker RA. 1999. Expression of MMP-2 and MMP-9, their inhibitors, and the activator MT1-MMP in primary breast carcinomas. J Pathol 189(2):161–168.

Lin EY, Gouon-Evans V, Nguyen AV, Pollard JW. 2002. The macrophage growth factor CSF-1 in mammary gland development and tumor progression. J Mammary Gland Biol Neoplasia 7(2):147–162.

Lin EY, Jones JG, Li P, Zhu L, Whitney KD, Muller WJ, Pollard JW. 2003. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. Am J Pathol 163(5):2113–2126.

Lund LR, Romer J, Thomasset N, Solberg H, Pyke C, Bissell MJ, Dano K, Werb Z. 1996. Two distinct phases of apoptosis in mammary gland involution: Proteinase-independent and -dependent pathways. Development 122(1):181–193.

Lund LR, Romer J, Bugge TH, Nielsen BS, Frandsen TL, Degen JL, Stephens RW, Dano K. 1999. Functional overlap between two classes of matrix-degrading proteases in wound healing. Embo J 18(17):4645–4656.

Marshman E, Streuli CH. 2002. Insulin-like growth factors and insulin-like growth factor binding proteins in mammary gland function. Breast Cancer Res 4(6):231–239.

Mudgett JS, Hutchinson NI, Chartrain NA, Forsyth AJ, McDonnell J, Singer II, Bayne EK, Flanagan J, Kawka D, Shen CF, Stevens K, Chen H, Trumbauer M, Visco DM. 1998. Susceptibility of stromelysin 1-deficient mice to collagen-induced arthritis and cartilage destruction. Arthritis Rheum 41(1):110–121.

Parks WC. 1999. Matrix metalloproteinases in repair. Wound Repair Regen 7(6):423–432.

Pilcher BK, Dumin JA, Sudbeck BD, Krane SM, Welgus HG, Parks WC. 1997a. The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. J Cell Biol 137(6):1445–1457.

Pilcher BK, Dumin JA, Sudbeck BD, Krane SM, Welgus HG, Parks WC. 1997b. The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. J Cell Biol 137(6):1445–1457.

Polette M, Birembaut P. 1998. Membrane-type metalloproteinases in tumor invasion. Int J Biochem Cell Biol 30(11):1195–1202.

Puente XS, Pendas AM, Llano E, Velasco G, Lopez-Otin C. 1996. Molecular cloning of a novel membrane-type matrix metalloproteinase from a human breast carcinoma. Cancer Res 56(5):944–949.

Radisky DC, Hirai Y, Bissell MJ. 2003. Delivering the message: Epimorphin and mammary epithelial morphogenesis. Trends Cell Biol 13(8):426–434.

Rudolph-Owen LA, Matrisian LM. 1998. Matrix metalloproteinases in remodeling of the normal and neoplastic mammary gland. J Mammary Gland Biol Neoplasia 3(2):177–189.

Sapi E. 2004. The role of CSF-1 in normal physiology of mammary gland and breast cancer: An update. Exp Biol Med (Maywood) 229(1):1–11.

Ueno H, Nakamura H, Inoue M, Imai K, Noguchi M, Sato H, Seiki M, Okada Y. 1997. Expression and tissue localization of membrane-types 1, 2, and 3 matrix metalloproteinases in human invasive breast carcinomas. Cancer Res 57(10):2055–2060.

Wiseman BS, Sternlicht MD, Lund LR, Alexander CM, Mott J, Bissell MJ, Soloway P, Itohara S, Werb Z. 2003. Site-specific inductive and inhibitory activities of MMP-2 and MMP-3 orchestrate mammary gland branching morphogenesis. J Cell Biol 162(6):1123–1133.